DO TISSUES SUPPRESS RADIATION-INDUCED CANCER?

A HYPOTHESIS RELATING CARCINOGENESIS, BYSTANDER EFFECTS AND GENOMIC INSTABILITY

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ABSTRACT

It is now well-established that cell growth, differentiation, and death are directed in large part by interactions with the microenvironment, which consists of cell-cell interactions, extracellular matrix and growth factors. This review discusses the evidence that an important consequence of such communication is the elimination of abnormal cells and the inhibition of neoplastic behavior. Recent studies indicate that radiation exposure induces a coordinated multicellular damage response in normal tissues that is characterized by remodeling of the microenvironment. We propose that radiation-induced bystander effects and genomic instability are actually manifestations of this homeostatic process. Bystander effects, found predominantly following low doses or non-homogenous radiation exposures, are evidence of indirect and direct communication that modulates cellular repair pathways and death programs. Genomic instability is evidenced following relatively high doses of ionizing radiation that persistently disrupt cell communication via the microenvironment, leading to the accumulation of aberrant cells. Transforming growth factor- is a key player in this program by acting as an extracellular sensor of damage, orchestrating multicellular damage responses that eliminate abnormal cells. These effects have been demonstrated both *in vitro* and *in vivo*. Understanding that radiation exposure results in tissue and organ responses requires re-evaluation of both radiation dose and risk.

Ionizing radiation delivered at high doses represent a well-established carcinogen in many tissues in both humans and animals. Radiation has a well-defined physical basis for action, a statistical probability of total and specific chemical events that are generally thought to result in damage to individual exposed cells at the time of irradiation. Studies of carcinogenic potential of ionizing radiation have commonly focused on initial DNA damage, which, if improperly repaired, can result in mutations or chromosome damage, some of which may lead to neoplastic transformation, others to cell death. Consequently, the nucleus is considered to be the major target of ionizing radiation damage. If ionizing radiation damages individual cells, one might argue that radiation response is the sum of individual cell responses. Such arguments result in the linear-no-threshold hypothesis that suggests that each unit of exposure or cellular damage results in an increase in radiation risk.

Alternatively, new data and reconsideration of earlier studies suggest that tissue response is greater than a sum of its cellular parts. We previously suggested that tissues respond to radiation, as they do to other damage, with a coordinated multicellular program in which individual cell contributions are directed towards repair of the tissue. For studies of processes that occur at the tissue level, the unit of response is multicellular interactions that are integrated via the microenvironment, rather than a cellular response integrated by the nucleus. In that there are now many studies demonstrating that normal tissue interactions inhibit the development of cancer through elimination of abnormal cells and suppression of neoplastic behavior, this perspective is particularly relevant to carcinogenesis as an endpoint of radiation risk.

Hypothesis

We propose the hypothesis that radiation-induced bystander effects and genomic instability are, respectively, positive and negative cellular manifestations of tissue programs of damage response. The bystander effect is a result of the intercellular communication necessary for coordinating multicellular responses leading, in part, to the elimination of abnormal cells. Suppression of this communication leads to accumulation of aberrant cells, which is evidenced as genomic instability. We will discuss studies that demonstrate these effects both *in vitro* and *in vivo* and evidence that transforming growth factor- (TGF-) plays a central role in this process. Finally, we propose that recognition that many of the responses related to radiation induced cancer are at the tissue level requires a re-evaluation of linear-no-threshold hypothesis.

Radiation-induced genomic instability and bystander effects

Radiation induced genomic instability has been described in a number of systems, both *in vivo* and *in vitro*, following exposure to ionizing radiation (1-3). Little and colleagues showed that irradiated cells transmit genetic instability to their progeny by a non-mutational mechanism and that cytoplasmic irradiation can result in increased mutations (4). Loss of genomic stability can be postulated to be a key feature in radiation induced cancer and result in the cascade of genetic change that results in the genetic diversity observed in most solid cancers (5, 6). Recent evidence suggests that cells surviving irradiation produce progeny that exhibit genomic instability at very high frequency unlikely to be due to conventional mutational changes (7). The frequency of genomic instability is more consistent with global changes in gene regulation, which are regulated in a large part by the microenvironment, than with gene mutation. Attempts to define the target and target size have suggested that the nucleus may be the target for the induction of genomic instability (8). Dynamic responses in gene expression represents a new concept in describing radiation's mode of action (9) that would also play an important role in genomic instability.

In the last decade "bystander effects", discussed in detail elsewhere in this volume, are when neighbors of irradiated cells acting as if they had been exposed and have been shown to involve cell-cell contact (10) and soluble signals (11). Low doses of radiation produced alterations in gene expression in a large number of different genes in most cells in a population (12, 13), which argues for the potential of irradiated cells to affect their neighbors (14). With the development of microbeams that make it possible to expose individual cells and exposure patterns and to identify individual cells have been exposed (15-17), it is now possible to monitor the response of each exposed cell and their neighbors. Such machines are capable of producing exposure patterns and exposure types that will be instrumental in addressing some very basic radiation biology questions.

Bystander effects are currently defined as biological changes in cells that do not have energy directly deposited in them. But a number of studies using transformation as an endpoint can also be construed as a type bystander effect. This effect can be clearly shown in cell culture by following the frequency of “initiation” using the morphological and behavioral benchmark of neoplastic behavior called transformation. Kennedy reported 20 years ago that the induction of transformation in the 10T1/2 cell culture model is not related to the radiation dose (18). The

number of transformed foci per dish was independent of the number of irradiated cells, thus leading to the conclusion that the transformed clones are not a direct consequence of the x-ray exposure. An additional explanation of the observation that increasing numbers of target cells leads to decreasing transformation frequency was raised in a series of thoughtful studies by Bauer and colleagues. Bauer has demonstrated that the ability of transform human and rodent fibroblasts is actively mediated by the non-transformed cells in a culture (reviewed in (19)). This process involves the selective ablation of transformed cells via apoptosis that is triggered by cytokines and reactive oxygen produced by non-transformed neighboring cells (20). Studies by Terzaghi-Howe also demonstrated the influence of normal cells using epithelial cell transformation. In a process called normalization, conditioned media from normal tracheal epithelial cells induced highly malignant rat tracheal carcinoma cells to undergo dramatic changes in morphology accompanied by a loss of anchorage independent growth (21). When cell culture conditions are manipulated it is clear that transformation is not related to carcinogen exposure in a fashion consistent with an induced rare-event, such as oncogenic mutations. Indeed, these studies indicate that transformation frequency may not even rely on the exposed population, but is rather a function of the response to the presence of normal, non-transformed cells.

A focus of radiation biology has been to use the understanding of genomic instability, bystander effects and the plasticity of the transformed phenotype to evaluate the risk of developing cancer in irradiated human populations. Before coming to a conclusion, one might ask, what do tissues have to do with genomic instability, bystander effects and the plasticity of the transformed phenotype? In other words, rather than asking how do cells become cancers, a more precise question may be how do tissues become tumors?

Cell phenotype is dictated by tissue microenvironment

Cells receive information about how they should behave from the microenvironment, which consists of other cells, insoluble extracellular matrix (ECM) proteins, soluble hormones and cytokines (22). Indeed, such epigenetic regulation is a dominant determinant of cell fate, as is evident from the more than 300 distinct cell types derived from the human genome. Both the recent success in cloning an entire animal from mature epithelial cell nuclei, and the remarkable plasticity of cell fate evidenced by multipotent stem cells support the contention that phenotype is

as much a function of environment as an expression of genotype (23). Experimental studies have led to the recognition that cells live or die by virtue of the presence of extrinsic survival signals such as ECM and growth factors, which suggests that there is little intrinsic 'will to live' attributable to the cell *per se* (24).

Specialized microenvironments, consisting of both insoluble ECM and soluble growth factors, play a pivotal role in normal tissue development, response to insults, and tissue function (22). Cell culture studies have demonstrated that proximity to a specialized ECM is a primary mediator of cell phenotype (25). The differentiated state of epithelial cells is poorly maintained in culture without an intact basement membrane (25) and *in vivo* development is disrupted when ECM deposition is inhibited (26-28). Epithelial cells are attached to a basement membrane, while stromal cells reside within the interstitial ECM. One physiological role for ECM is to sequester and concentrate growth factors in proximity to cell membranes. Growth factors also stimulate the production of ECM, that in turn serves to modulate epithelial cell growth. Signaling via ECM receptors influences the production of growth factors, while the association of cells with particular ECMs alters their response to growth factors and hormones. Cell adhesion in particular is critical to the ability of cells to behave in a tissue-specific fashion and has recently been identified as an avenue to modulate the neoplastic in human breast cells (29, 30). Conversely, the therapeutic benefit of -interferon in chronic myeloid leukemia is due in part to the re-establishment of cell-adhesion signals (31). Perturbation of this critical interaction negatively affects cell phenotype as has been widely demonstrated by the loss of function that occurs when cells are dissociated from tissues and cultured in the absence of ECM (32). Given the definitive evidence, beyond the scope of this review (33), that cell behavior is a consequence of the microenvironment, nothing seems to preclude the microenvironment from affecting the development of cancer.

Normal tissues suppress cancer

Quantitative studies in rodent models demonstrate that the number of cells initiated following either physical or chemical carcinogen exposure, far exceeds the number of tumors that develop in vivo (34-36). What then prevents us all from developing cancer, rather than the 1/8 incidence that spans 70+ years and the production of 10^{18} cells? A variety of studies suggest that expansion of an initiated population is actively opposed/suppressed by normal cells. Despite the

evidence of the clonogenic nature of cancer, tumor formation requires the complicity of normal cells and normal tissue interactions, all of which are mediated via the microenvironment. More importantly, tissues are also capable of actively inhibiting cancer formation (36-38). Perhaps the best recognized are the experiments discussed by Pierce in which carcinoma cells are induced to 'normalize' by virtue of their placement within developing embryos (39). Despite the presence of genetic sequence alterations, these cells behave appropriately in response to the overwhelming influence of the microenvironment and their normal neighbors. Pierce likened this to the process of differentiation that occurs via extracellular signaling in normal tissues and was among the first to propose that how the genome is controlled is as important as genetic change in cancer (39).

Abnormal tissues promote cancer

Pierce also proposed the corollary that carcinogenesis is a caricature of this process, in which the regulatory controls were disrupted. An example of this was identified by Schor and colleagues, who postulated that the inherited mutation in some familial breast cancers could disrupt tissue interactions in such a way that then provides a stimulus for initiated cells to exhibit more aggressive neoplastic behaviors (40, 41). This hypothesis arose from the description of fetal-like migratory behaviors in tumor fibroblasts. Surprisingly, skin fibroblasts from familial, but not spontaneous, breast cancer patients also showed this phenotype(42). In addition, the normal appearing tissue next to breast cancer also displays these changes (43). The frequency of first-degree relatives who exhibit the altered skin fibroblast phenotype is consistent with an inherited trait (44). Thus the increased probability of cancer in these individuals was postulated to be due not to the probability of acquiring epithelial mutations, but an increased potential for their persistence due to the presence of an abnormal stroma. Likewise, perturbation of cell adhesion molecules by diseased or genetically aberrant stromas early in neoplastic progression has also been suggested in hematopoeitic malignancies (45).

It has been suggested that dynamic interplay between tumor and stroma are evidence that tumors are 'wounds that does not heal' (46), but a wound environment may precede tumorigenesis. Wounding and chronic inflammation can both act as promoters, apparently by creating a favorable microenvironment for proliferation, a prerequisite for wound repair (reviewed in (47)). An important feature of wounding is the conversion of the stroma from a quiescent tissue with a very stable composition, to an activated state in which there is dynamic

remodeling of the microenvironment by resident and transient cells. Experiments in the 1960's by Fisher and colleagues showed that tumors metastasize preferentially to wound sites in parabiotic pairs of animals injected with invasive tumor cells (48). Experiments performed with Rous sarcoma virus showed that tumors formed preferentially at sites of injections or at distant wounds (49). In the Rous sarcoma model, TGF- can be substituted to promote tumor formation similar to wounding if injected directly into distal sites (50). Several transgenic oncogene models show preferential tumorigenesis at wound sites (51). Carcinogenesis is enhanced in experimental animal models of activated stroma induced by wounding (49), overexpression of platelet-derived growth factor (52), or misregulation of stromelysin (53, 54).

Since tissue pathology arises from fundamental disruption of orchestrated communication between cells and among different cell types, the influence of normal cells on neoplastic behavior is compromised in abnormal tissues. A variety of experimental manipulations of the microenvironment stimulate tumorigenesis. Mammary preneoplastic nodules are more tumorigenic when the tissue is disaggregated before transplantation, suggesting maintenance of tissue architecture suppresses neoplastic behavior even in explanted tissue (55). Epithelial cells from irradiated rat thyroid gland, mammary tissue or liver and transplanted to unirradiated fat pad have a much higher transformation frequency than the cancer frequency observed in the normal cellular environment, suggesting that disruption of the intact tissue and heterotypic microenvironment played an important role in expression of radiation induced cancer (56). When adult mouse mammary epithelium is combined with salivary gland mesenchyme, it not only undergoes ductal branching patterns typical of salivary gland (57), but also gives rise to a greater incidence of tumors (58). These studies are evidence that neoplastic potential, rather than being fixed in the target cells, is highly responsive to tissue factors.

The role of tissues in radiation-induced cancer

The forces that inhibit or stimulate expression of tumorigenic potential, i.e. selection, are probably more critical in determining cancer frequency than initiation (59). Radiation exposure changes the expression of many genes involved in tissue processes such as proteases, growth factors, cytokines and adhesion proteins, which supports the view that radiation exposure compromises tissue integrity by altering the flow of information among cells (60). If the unit of function is taken into account, i.e., tissue, it becomes evident that many of these events are likely

directed to the good of the whole, rather than the part, i.e., the cell. But understanding which responses provide a benefit that leads to reestablishment of homeostasis and which are detrimental and contribute to radiation late effects is not understood at this time. For example, irradiated mouse mammary gland undergoes rapid remodeling of the microenvironment characterized by changes in ECM and activation of latent TGF- (61, 62). Functional confirmation of TGF- as a mediator of tissue response to ionizing radiation was obtained by treating animals with TGF- neutralizing antibodies before irradiation (63). TGF- has profound effects on both epithelial and mesenchymal cell growth, differentiation and apoptosis (64). It has been proposed that TGF- mediates the establishment of radiation fibrosis (65), but its specific contribution tissue repair versus chronic disease are not fully understood. Differential mRNA display revealed that cell adhesion, signal transduction and gene transcription and translation were prominent pathways induced in malignant cells exposed to normal cell conditioned media containing TGF- from during their reversion to normal behavior (66). Cell adhesion in particular is critical to the ability of cells to behave in a tissue-specific fashion and has recently been identified as an avenue to modulate the neoplastic in human breast cells (29, 30). Thus gene expression by irradiated cells and tissues may have many consequences.

Since aspects of radiation-induced remodeling, such as TGF- , parallel those in dermal wound healing, the effects of ionizing radiation on tissue microenvironment may be similar to those agents resulting in activated stroma that in turn fosters neoplastic behavior (67). Based on the known carcinogenic risk of radiation exposure, the dependence of cells on extracellular signaling and the rapid remodeling observed in irradiated tissue, we asked whether microenvironment remodeling contributes to the risk of radiogenic carcinogenesis (68). Syngeneic mice were cleared of epithelia were irradiated so that the mammary stroma, rather than the intact tissue, and were then transplanted with unirradiated mammary epithelial cells (69). These cells are non-tumorigenic in nude mice, or when transplanted subcutaneously in syngeneic hosts, but both p53 alleles are mutated, conferring neoplastic potential. Tumor incidence was increased 4-fold compared with sham-irradiated hosts when host animals were irradiated at a dose rate of 23 cGy/min for a total dose of 4 Gy 3 days prior to transplantation. Tumors were also significantly larger and arose more quickly in fat pads in irradiated hosts. Since tumors formed only in fat pads on the irradiated side of hemi-body irradiated animals, the influence of the irradiated tissue dominated over systemic effects. These data indicate that radiation-induced

changes in the stromal microenvironment can contribute to neoplastic progression *in vivo*. Remodeling of the extracellular matrix and TGF- activation are detectable following whole body exposures of 0.5 Gy and 0.1 Gy respectively. (63); the effect of dose and dose rate in in this model. We postulated that radiation-induced changes in microenvironments are evidence of an additional class of carcinogenic action, distinct from those leading to mutations or proliferation (68).

These experiments suggest that bystander phenomena also exist *in vivo*, in that the products

of irradiated cells significantly alter the phenotype of unirradiated cells. Carcinogen-induced microenvironments are not necessarily mutagenic or mitogenic *per se*. Rather, changes in the microenvironment may promote neoplastic behavior by changing gene expression, disrupting normal cell functions regulated through cell-cell contact through cell-ECM interactions and growth factor signaling. Greenberger and colleagues have also proposed a model of indirect - irradiation leukemogenesis based on co-cultures of heavily irradiated bone marrow stromal cell lines that selectively bound M-CSF receptor positive unirradiated hematopoietic progenitor cells resulting in selection of tumorigenic subclones (reviewed in (70)). Such studies support the conclusion that radiation has global and persistent consequences on stromal function, which in turn can influence the expression of neoplastic potential. This is also evident in chemical carcinogenesis. Hodges and colleagues showed that cultured carcinogen-treated stroma recombined with normal bladder epithelium produce neoplastic changes in epithelial morphology (71). Zarbl and colleagues found that *Hras*1 gene mutations in mammary tumors from *N*-nitroso-*N*-methylurea treated rats arose from cells with preexisting *Hras*1 mutations that had occurred during early development (72). Thus, although clearly mutagenic in its own right, *N*-nitroso-*N*-methylurea exposure apparently led to the expansion and neoplastic progression of *Hras1*-mutation containing populations.

In the mammary gland studies discussed above, the interplay between epithelial target cells and the irradiated stroma demonstrates the deleterious effects of radiation on tissue mechanisms that suppress cancer. In the elegant studies of Clifton and colleagues, irradiated epithelial cells transplanted to non-irradiated, but abnormal stroma, quantitatively demonstrate that radiation-induced epithelial cell initiation is a frequent event (36). Similarly Ullrich and Ethier used transplantation to an unirradiated host to reveal the neoplastic potential of radiation and chemical carcinogen exposed mammary epithelial cells (73). Nonetheless, if the same irradiated populations are left *in situ*, subject to normal tissue interactions, cancer is efficiently suppressed. Disruption of the tissue interactions that effectively suppress neoplastic behavior is a new activity of radiation as a carcinogen.

Bystander effects and cancer induction in vivo

The hypothesis that "radiation induces DNA damage, DNA damage induces mutations and mutations cause cancer" can be challenged by the evidence of bystander effects and genomic

instability (74). Transformation assays attempt to measure events linking radiation and cancer one step further by showing a relationship between dose and the frequency of the 'transformed' phenotype, an endpoint related to cell proliferation, adhesion and/or morphology. But in reality, our mechanistic understanding of the processes involved in neoplastic transformation in cell culture and the maintenance of the transformed phenotype over many generations of cell replication is woefully incomplete. There is good evidence in many systems that regulation of cell transformation is related to both cell/cell communication and cell/matrix communication, that could be conceivably be construed as bystander effects. Since transformation is postulated to be the cell culture equivalent of initiation, one may question whether risk can really be based on what we understand of the nature of the 'initiating' events in carcinogenesis. Thus, after establishing that bystander effects can be easily demonstrated in tissue culture systems and that they seem to play a role in the induction of genomic instability, it is essential that they be demonstrated *in vivo* and ultimately in human systems. Without such evidence, the biological significance of bystanders would have limited impact on risk.

There are a number of studies suggesting that there are clastogenic factors released into the blood following whole body irradiation which are capable of causing chromosome damage in cells *in vitro* (75, 76), although other studies failed to demonstrate radiation induced clastogenic factors (77). Research evaluating the influence of partial organ irradiation has also demonstrated that exposure of a part of the lung can result in cytogenetic damage in cells that are outside the field of the radiation exposure (78). Both of these types of experiments suggest the release of factors that can be transported to other regions of the body and cause damage and can perhaps increase the risk to non-exposed tissue. This response is related to soluble factors and is not the same as observed in tissue culture where the presence of gap junctions (79) and direct cell/cell communication are necessary for induction of the bystander effect; Azzam, 1998 #2626]. Examples of the influence of cell/cell communication have been demonstrated in organized cells in tissue culture as well as in isolated tissue preparations such as tracheal epithelium. These cells were exposed to low doses of alpha particles. The dose was regulated to result in only a small fraction of the cells being traversed by alpha particles. The induction of p53 was seen in all the epithelial cells suggesting that the cells were responding as a unit (80).

Research with internally deposited radioactive materials like ^{239}Pu suggested that bystander effects exist *in vivo* since more cells responded with the production of chromosome

aberrations than were traversed by alpha particles (81, 82). Chinese hamsters were injected with $^{239}PuO_2$ or ^{239}Pu citrate. This alpha emitter is concentrated in the liver and results in a chronic low dose-rate exposure to this organ. The $^{239}PuO_2$ particles produced with a range of particle sizes (0.14-0.84 μm) and were injected at the same total activity. This resulted in the same total dose to the liver but a very large differences in local dose and dose-rate delivered to cells that were physically located near the particles. The dose-response relationships for induction of chromosome aberrations following exposure to each of the particle sizes and the ^{239}Pu citrate were not significantly different (81). Thus, there was no change in the frequency of unstable chromosome aberrations as a function of local dose distribution. These data suggest that all the cells in the liver were at the same risk for the induction of the initial chromosome damage even though a small fraction of the total liver cell population was exposed to alpha particles following the injection of the animals with the large particle sizes.

The cumulative liver cancer incidence as a function of time after injection was also determined as a function of total dose. The results of these studies also illustrated that the time of onset, dose and cancer incidence were not modified as a function of particle size or number of cells "hit" by alpha particles. Animals injected with the citrate form of the ^{239}Pu had a shorter latency period for liver tumor production. However, at levels of $^{239}PuO_2$ or ^{239}Pu citrate that did not result in life shortening the survival and total cancer incidence was the same (82). These studies offer direct evidence that the liver was responding as a total unit as far as cancer risk is concerned. The risk for the induction of both chromosome aberrations and cancer in this system is thus related to the total energy and total dose to the organ, not the local distribution or local dose to individual cells or the number of cells traversed by the alpha particles.

Microenvironment mediators: TGF- in carcinogenesis

When injury leaves tissue intact, such as inflammation, UV and ionizing radiation, what molecular mechanisms record or 'sense' the damage? Intracellular mechanisms for sensing DNA damage are thought to dictate individual cell responses, such as growth delay and DNA repair. If so, one might expect that the response of a given cell type to a given dose of radiation to be invariant since the amount of DNA damage is due to the deposited energy. This is not so, in that the presence of cytokines and ECM dictates whether the irradiated cell lives or dies, proliferates or stops (83, 84). Furthermore, damaged cells may not be capable of contributing to the

repair/reconstitution of tissue and may opt for self-destruction via apoptosis as the best solution for maintaining tissue integrity (85). One might postulate that sensors beyond individual cells have evolved that can register tissue damage and producing a signal that will recruit non-damaged cells to facilitate recovery.

The flow of information both locally between cells in tissues, and distantly between organs is mediated in large part by cytokines (86). TGF- is one such critical factor that orchestrates multi-cellular responses to damage via effects on proliferation, apoptosis, ECM composition, growth factor production, chemotaxis and immune function (87, 88). Although TGF- accumulates at wound sites it appears to have evolved to repair quickly, rather than restore, tissue integrity, as evidenced by improved healing if its effects are attenuated (89, 90). These observations lead to the notion that TGF- is poised for an exuberant response that can also become a liability. Our studies have shown that ionizing radiation elicits rapid and persistent TGF- activation (63). Other DNA damaging agents, such as cis-platinum (91) and alkylating agents (92), also induce TGF- activity.

In the cultured fibroblast transformation model, Bauer has described three distinct, but competing, roles for TGF- (reviewed in (93)). Although TGF- helps maintain the transformed state, it also enables non-transformed neighbors to recognize the transformed cells, thereby triggering an apoptosis-inducing response. In the experiments by Terzaghi-Howe, TGF- produced by the differentiated normal epithelial cells inhibits carcinogen-altered cells (21). If this control system acts in vivo as efficiently as it does in vitro, tumor formation should require the establishment of resistance mechanisms directed against intercellular induction of apoptosis. Indeed when cells from established tumors were tested for inhibition by normal cells in culture, they fail to be influenced (94).

This role is supported by transgenic mouse models that have targeted expression of constitutively active TGF- . Epidermally-targeted over expression of TGF- inhibited the establishment of benign early skin tumors following carcinogen exposure, and likewise MMTV-promoter driven expression of TGF- suppresses the formation of mammary tumors induced by transgenic induction of an oncogene (95). However, continued exposure to TGF- increases the frequency with which the benign tumors convert to malignant spindle cell carcinomas (96, 97). In a similar model, genetic inactivation of TGF- signaling by overexpression of a dominant/negative TGF- type II receptor in the mouse epidermis also accelerates tumor

progression with a 6-fold increase in malignant conversion frequency from benign papillomas to carcinomas (98). A spindle

cell carcinoma reflects an epithelial to mesenchymal transition that occurs during normal development but is also induced by TGF- to produce invasive metastatic carcinoma cells (99). Nonetheless carcinogen treatment of *TGF- 1 null* heterozygotes reveals that TGF- insufficiency promotes tumorigenesis (100). Together these data suggest that TGF- action is two-edged: TGF- can suppress the establishment of tumorigenesis but its continued elevation, or perturbation of its signaling, promote malignant behavior.

It is highly relevant to TGF- role in carcinogenesis, that keratinocytes cultured from *TGF- null* animals have greatly elevated genomic instability as indicated by gene amplification assay (101). The frequency of instability could be reversed by addition of low levels of exogenous TGF- . Aneuploidy, chromosome breaks, and malignant transformation of v-ras(Ha) transduced primary *TGF- 1 null* keratinocytes were also suppressed by exogenous TGF- . From these studies, the authors conclude that genomic instability is a mechanism accelerates tumor progression in tumors harboring defects in TGF- signaling (102).

It has been suggested that the control of the genome and gene expression, not changes in DNA base sequence, may be in a large part responsible for genomic instability (8). It is perhaps more surprising that an extracellular factor regulates genome integrity, a task usually associated with cellular gatekeepers like p53. At present, little is understood of the processes resulting in genomic instability and in the maintenance and transmission of the phenotype over many generations. One might ask how the absence of TGF- contributes to, or amplifies, genomic instability. Is it due to defective growth regulation, allowing damaged cells to proliferate when they should be blocked in the cell cycle to repair damaged DNA? Or is it possible, as suggested by the transformation studies described above, that TGF- selectively impedes the survival of aberrant cells? The fact that exogenous TGF- corrected the defect in *TGF- null* cells suggests either that genomic instability is a reversible phenotype, or that the absence of TGF- reveals the presence of unstable cells that would otherwise be eliminated, i.e. TGF- alters the detection of instability. If so, rather than considering genomic instability as an induced process in irradiated cells, the phenomena may reflect insensitivity to multicellular signals that should inhibit the survival of abnormal cells.

SUMMARY

Organisms are comprised of cells that interact as functional units, the purpose of which is to maintain tissue function; as a consequence, individual cells are remarkably redundant and expendable. As a consequence of multi-cellular organization, abnormal cell behavior is not tolerated and most cells have low thresholds to undergo apoptosis if damaged. (The exceptions include highly differentiated, short-lived cells, such as transit cells of the small intestine, or highly-differentiated cells with low proliferative potential like neurons.) This trigger may even extend to siblings via either lineage heritable defects, or a conditional state, or via communication of the damage response. In addition to the cellular damage threshold, extracellular sensors of damage exist that stimulate the 'altruistic suicide' of defective cells. In a non-transformed multicellular population, we propose that very low doses of ionizing radiation could stimulate this function that eliminates abnormal cells. This is detected in experimental animal models using non-homogeneous radiation exposure such as the plutonium particle size effect (or lack thereof) in liver. In cell culture models, detection of this program is evidenced by the effect of normal cells on suppressing transformation frequency and the response of non-irradiated cells to the presence of irradiated cells. The latter is called the bystander effect, and is most readily characterized in non-homogeneous radiation exposures such as alpha-particles but it is also influenced by the model features such as species, cell type, degree of normalcy, and culture conditions. When all cells are irradiated with significant dose, this function is inhibited since the irradiated cells lose their ability to interact with the microenvironment and other cells. Under these circumstances, abnormal cells accumulate, which is measured as endpoints such as genomic instability and transformation in cell culture models, and which contributes to the development of neoplasia in vivo.

Implications for risk

With demonstration of radiation induced bystander effects and the role of these effects on cancer process it is important to again re-examine the concept of dose and target size. If the biological response related to the induction of cancer is the whole organ rather than single cells then radiation dosimetry should be based on the mass of the whole organ and the total dose or energy absorbed in the organ. One of the major problems associated with dose calculations and the extrapolation of dose to risk is to know the proper mass of the target assumed for the dose calculation. The mass can be taken as a subcellular structure, the cell nucleus, the whole cell, a tissue, the whole organ or the total body. If cells respond independently then the mass should be

a cell, if the tissue responds then the mass considered should be the tissue etc. In the past, especially for non-uniform high LET radiation exposures dose has been calculated for individual cells or cell nuclei (103). The observations of bystander effects and genomic instability makes it difficult to determine the appropriate target size or mass to use for dose calculations. For example, in calculation of dose and subsequently estimating risk from radon exposure the size of the target has been taken as the lung epithelial cells that line the airway, since this is the target where the energy is deposited and the site of the tumors (104). If the carcinogenic response to radon exposure is not dependent on the epithelial cells alone but is dependent on the dose and response of all the cells in the lung the mass to be used in dose calculation would be increased. This increase in mass would of course result in a decrease in the dose from radon per unit of activity. Such a procedure would greatly reduce the "risk" from radon in homes (104) and the fraction of the total background radiation dose that is assigned to radon (105).

In this paper, we have reviewed some of the extensive data that suggests that tissues, not cells, are the target for carcinogenesis in that the behavior of all cells, including many neoplastic cells, is a function of their environment. The cellular response to radiation is part of a multicellular program that is a response to the perturbation of the homeostatic state of the tissue, and in large part is directed toward restoring that state. There is evidence that exposure to high doses of ionizing radiation impedes this process, which can lead to the persistence of genomic unstable cells, that may drive neoplasia. Whether low doses of radiation also promote carcinogenesis by this means remains to be determined. It is conceivable that normal tissues interactions are intact, or possibly stimulated, at low doses and can effectively eliminate potential cancer cells. Thus it is important to consider the whole tissue response in calculation of dose and estimating risk.

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